69. (New) The method of Claim 67, further comprising the step of (e) coexpressing said population of altered light chain variable region encoding nucleic acids with a heavy chain variable region encoding nucleic acid so as to produce a diverse population of altered heteromeric variable regions.

70. (New) The method of Claim 67, wherein said synthesizing comprises chemically synthesizing.

71. (New) The method of Claim 67, wherein said acceptor is human.

REMARKS

Claims 42-51 are pending, and stand rejected in the present application. Claims 52-71 have been added. The Examiner has rejected Claims 42-51 on the following grounds:

1. Claims 42-51 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly lacking enablement.
2. Claims 42-51 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly lacking support in the specification with respect to the phrase "three complementarity-determining regions as defined by the combined definitions of Kabat and Chothia".
3. Claims 42-51 stand rejected under 35 U.S.C. § 112, second paragraph as allegedly indefinite.
4. Claims 42-51 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Deng *et al.* [Canadian Patent Application 2,125,240 A1].

Applicants believe that the following remarks traverse the Examiner's rejection of the Claims. These remarks are presented in the same order as they appear above.

1. The Claims are Enabled

The Examiner has rejected Claims 42-51 under 35 U.S.C. § 112, first paragraph as allegedly not being enabled, as they encompass both "mixing oligonucleotides in any order" (in reference to step (c) of claims 42 and 47) and they encompass "oligos that code for only

framework regions or only CDRs, and oligos that encode CDRs in any order" (page 2 of the Final Office Action mailed 10/23/01). Additionally, the Examiner appears to suggest that the claims encompass heavy or light chains wherein the CDRs are in any random order (page 2 of the Final Office Action mailed 10/23/01).

Applicants respectfully traverse this point of rejection. As noted in the accompanying Declaration of Dr. Watkins pursuant to 37 C.F.R. § 1.132, "overlapping oligonucleotides" overlap due to hybridization of complementary sequences, which are present at the ends of adjacent oligonucleotides (see paragraph 3 of Dr. Watkins' Declaration). As noted by Dr. Watkins, hybridization of oligonucleotides occurs in an orderly manner. Moreover, the specification as filed provides information that make it clear that the heavy and light chains of an antibody variable region each have four FRs and three CDRs, in a defined order, based on the amino acid sequence. The Examiner is referred to page 9, line 22 through page 10, line 2 of the specification as filed:

> "As used herein, the term "framework region" is intended to mean each domain of the framework that is separated by the CDRs. Therefore, for the specific example of a heavy chain variable region and for the CDRs as defined by Kabat et al., framework region 1 corresponds to the domain of the variable region encompassing amino acids 1-30; region 2 corresponds to the domain of the variable region encompassing amino acids 36-49; region 3 corresponds to the domain of the variable region encompassing amino acids 66-94, and region 4 corresponds to the domain of the variable region from amino acids 103 to the end of the variable region."

This information, together with the Kabat *et al.* CDR definitions shown in Table 1 (page 9 of the specification as filed), would thus result in an exemplary heavy chain variable region made up of framework region 1 (amino acids 1-30), followed by CDR1 (amino acids 31-35), followed by FR2 (amino acids 36-49), followed by CDR2 (amino acids 50-65), followed by FR3 (amino acids 66-94), followed by CDR3 (amino acids 95-102), followed by FR4 (amino acid 103 through to the end of the heavy chain variable region). One of skill in the art would understand from this information that a heavy or light chain variable region is made up of four framework regions and three CDRs, in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 (see paragraph 4 of Dr. Watkins' declaration).

Although the above exemplary heavy chain variable region has been described with reference to the Kabat *et al.* definitions for CDRs, one of skill in the art could readily use the information in Table 1 to delineate the boundaries of the four framework regions and the

three CDRs in a heavy or light chain variable region based on the combined definitions of Kabat and Chothia.

Without Acquiescing to the Examiner's rejection, but in consideration of the business interests of the Applicants and in order to further the prosecution, and hereby expressly reserving the right to prosecute the original (or similar claims), Applicants have amended Claims 42 and 47 to further define one embodiment of the invention. Specifically, Applicants have amended the Claims to specify that the modified CDR encoding oligonucleotides from the second population of oligonucleotides also contain sequences capable of hybridizing to the "adjacent" framework region, *i.e.* they hybridize to the first population of oligonucleotides. Thus, in the presently claimed embodiment, the modified framework region and the modified CDR are adjacent to one another. Applicants reiterate that they are expressly reserving the right to claim and prosecute other embodiments in future applications (non-limiting examples of such embodiments include an embodiment in which the modified CDR(s) are not adjacent to the modified FR(s) and an embodiment in which the oligonucleotides overlap in CDR sequences).

2. The Claims are Supported

The Examiner has rejected Claims 42-51 under 35 U.S.C. § 112, first paragraph because the response of 8/20/01 (which was mailed 8/15/01) does not state where in the application as originally filed support for the phrase "three complementarity-determining regions as defined by the combined definitions of Kabat and Chothia" can be found (page 3 of the Final Office Action mailed 10/23/01).

Applicants respectfully direct the Examiner's attention to page 44, line 24 through page 45, line 12 of the application as filed:

> "Additionally, the methods for conferring donor CDR binding affinity onto an antibody acceptor variable region framework are applicable for grafting CDRs as described by Kabat et al., *supra*, Chothia et al., *supra* or MacCallum et al., *supra*. The methods similarly can be used for grafting into an acceptor framework overlapping regions or combinations of CDR as described by these authors. Generally, the methods will graft variable region CDRs by identifying the boundries described by one of the CDR definitions known in the art and set forth herein. However, because the methods are directed to constructing and screening populations of CDR grafted altered variable regions which incorporate relevant amino acid position changes in both the framework and

> CDR regions, and such variations can, for example, compensate or augment amino acid changes elsewhere in the variable region, the exact boundry of a particular CDR or set of variable region CDRs can be varied. Therefore, the exact CDR region to graft, whether it is the region described by **Kabat et al., Chothia et al. or MacCallum et al., or any combination thereof**, will essentially depend on the preference of the user." (emphasis added)

Applicants respectfully submit that this supports the use, in the present embodiment of the invention, of the combined definition of Kabat and Chothia to define CDR (and thus also framework) residues.

3. The Claims are Definite

The Examiner has rejected Claims 42-51 under 35 U.S.C. § 112, second paragraph as being allegedly indefinite for two reasons: (a) The Examiner has found insufficient antecedent basis for the limitation "first reference nucleic acid sequence" in claims 42 and 47 (page 4 of the Final Office Action mailed 10/23/01). Without acquiescing to the Examiner, and in consideration of the business interests of the Applicants, and solely to further the prosecution of the application, and expressly reserving the right to prosecute the same or similar claims in a future application, Applicants have amended Claims 42 and 47. Applicants respectfully submit that the instant amendment renders the rejection moot, and respectfully request that the Examiner withdraw this rejection. And (b) The Examiner has found claims 42-51 to be allegedly indefinite for reciting the term "potion" [sic] because the exact meaning of the term is allegedly not clear (page 4 of the Final Office Action mailed 10/23/01). Applicants respectfully disagree.

As noted in paragraph 5 of his declaration, Dr. Watkins has identified sections of the specification that support the use of the term "portion", in reference to a sequence or a molecule, to refer to a "part or a fragment" of the molecule or sequence (see Dr. Watkins' Declaration at paragraph 5). For example, page 10, lines 10-18 of the specification as filed:

> "As used herein, the term "donor" is intended to mean a parent antibody molecule or fragment thereof from which a **portion** is derived from, given or contributes to another antibody molecule or fragment thereof so as to confer either a structural or functional characteristic of the parent molecule onto the receiving molecule. For the specific example of CDR grafting, the parent molecule from which the grafted CDRs are derived is a donor molecule." (emphasis added).

Similarly, on page 10, lines 24-27 of the specification as filed:

> "As used herein, the term "acceptor" is intended to mean an antibody molecule or fragment thereof which is to receive the donated **portion** from the parent or donor antibody molecule or fragment thereof." (emphasis added).

And on page 11, lines 4-10 of the specification as filed:

> "A "variable region" when used in reference to an antibody or a heavy or light chain thereof is intended to mean the **amino terminal portion** of an antibody which confers antigen binding onto the molecule and which is not the constant region. The term is intended to include functional fragments thereof which maintain some of all of the binding function of the whole variable region." (emphasis added).

And finally, on page 23, lines 21-25 of the specification as filed:

> "The replacement of each position can occur in the context of other donor CDR amino acid positions so that a significant **portion of the CDR** maintains the authentic donor CDR sequence, and therefore, the binding affinity of the donor CDR." (emphasis added)

These excerpts make it clear that a "portion" of a molecule refers to a part of that molecule (Dr. Watkins Declaration at paragraph 5). Thus, in the case of a protein, it would refer to a part of that protein molecule, and in the case of a nucleic acid molecule, it would refer to a part of that nucleic acid molecule.

Importantly, one of skill in the art would understand that the sequences encoding the portion(s) of the adjacent framework region(s) on the oligonucleotides of the second population permits their annealing and hybridization to the corresponding sequences on the oligonucleotides of the first population, and therefore "portion" does not refer to a single nucleotide.

4. The Claims are Not Obvious

Claims 42-51 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Deng *et al.* (Canadian Patent Application 2,125,240 A1). Applicants disagree. The Examiner has characterized Deng *et al.* as teaching a method of producing a population of heavy or light chains wherein the CDRs are randomized and the framework regions are randomized (page 5 of the Final Office Action mailed 10/23/01). The Examiner states that the method of Deng *et al.* involves the chemical synthesis of oligos, and framework residues that are randomized are those that differ between the murine and human. The Examiner has noted that Deng *et al.* does not specifically teach that the reference sequences are in electronic form (page 5 of the Final Office Action mailed 10/23/01).

Applicants cannot agree. There are numerous points on which Deng *et al.* differs from the pending claims.

First, none of the examples appear to be directed to the synthesis of antibodies with the simultaneous modification of CDR and FR residues. Although Deng *et al.* report some FR mutations after synthesis involving spiking CDR sequences, the legend to figure 4 (see page 6, lines 16-18 of Deng *et al.*) states "Some deletions (indicated by D) and framework mutations, **thought to be DNA synthesizer errors**, were also observed." (emphasis added). Applicants argue that the introduction of random errors during oligonucleotide synthesis are clearly not the same as the introduction of a modification (a different, or changed amino acid) at one or more framework region positions, wherein said framework positions that are changed are selected from among those which differ between a reference acceptor sequence and the corresponding positions of a reference donor sequence.

Second, in contrast to the controlled spiking described by Deng *et al.*, new claims 62 and 67 specify that the changed amino acids present in the modified CDR encoding population and the modified framework region encoding population were introduced through the use of codon-based mutagenesis. Applicants note that support for the use of codon-based mutagenesis to rapidly and efficiently produce a large number of alterations in a known amino acid sequence, or for generating a diverse population of variable or random sequences can be found from page 31 (line 30) through to page 32 (line 16) in the specification as filed.

Third, the Examiner characterizes Deng *et al.* as teaching that the framework residues which are altered are those which differ between the murine and the human, and cites pages 22-23 of Deng *et al.* (page 5 of the Final Office Action mailed 10/23/01). Pages 22-23 of Deng *et al.* (specifically page 22, line 34 - page 23, line 2 of Deng *et al.*) describe replacing murine framework 1, 2 and 3 regions of V_H of a particular scFv with partially randomized frameworks from the human antibody NEW. It is stated that the residues selected for randomization were those "which frequently differ in murine and human V_H frameworks". The choice of frameworks "which frequently differ" in murine and human V_H frameworks is not the same as selecting residues based on *actual* differences between two reference sequences. Rather, selecting residues based on frequent differences between frameworks suggests the use of consensus sequences, derived from a collection of human and murine sequences, rather than a direct comparison of two particular reference sequences. In this regard, Deng *et al.*

appear to teach away from the instantly claimed embodiment. Furthermore, the example of Deng *et al.* beginning on page 22 (*i.e.* Example 3 of Deng *et al.*) is directed solely to the modification of framework residues of a heavy chain variable region. As noted above, the instantly claimed embodiments are directed to the simultaneous modification of at least one CDR (or portion thereof) and at least one adjacent FR (or portion thereof), in heavy chains (Claims 42, 52 and 62) and light chains (Claims 47, 57 and 67).

Finally, Deng *et al.* describe gene assembly through the ligase chain reaction or through standard T4 ligations. This is in contrast to the use of a polymerase to assemble the overlapping oligonucleotides, as described in the instant specification as filed (page 48, line 11). This distinction is specified in new claims 52 and 57.

Thus, for the reasons set forth above, Applicants are unable to find teaching in Deng *et al.* for each and every element of the present claims (which represent but one embodiment of the present invention). Specifically, Deng *et al.* fails to teach the generation of populations of nucleic acids encoding simultaneous framework changes and CDR changes, wherein the changes are assessed based on specific donor and acceptor reference sequences, and the specific framework residues which are altered are selected based on the residues which differ between the donor and acceptor reference sequences. The Examiner also references page 13, lines 12-14 of Deng *et al.* (see page 5 of the Final Office Action mailed 10/23/01), where it is stated "Therefore, not only can the CDRs be randomized but also the frameworks.". As noted above, this does not teach the generation of populations of nucleic acids encoding simultaneous framework changes and CDR changes, wherein the changes are assessed based on donor and acceptor reference sequences, and the specific framework residues which are altered as selected based on the residues which differ between the donor and acceptor reference sequences. Rather, the sentence from Deng *et al.* noted above appears to merely reiterate that CDRs can be altered, as can FRs.

The Examiner's opinion that it would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have the first and second reference sequences to be in electronic form (see page 5 of the Final Office Action mailed 10/23/01), fails to make up for the above-noted deficiencies in Deng *et al.* Furthermore, the Examiner's opinion is not "evidence", as the Examiner is not one of skill in the art (under law), and the Examiner's opinion on what one skilled in the art might believe

does not count. *In re Rijckaert*, 9 F.3d 1531, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993) ("[T]he examiner's assumptions do not constitute the disclosure of the prior art."). Of course, if the Examiner has knowledge of relevant facts which are used to make the rejection, the Examiner is free to use those facts - but only if submitted in the form of an affidavit. See 37 CFR 1.107(b). In the present case, the Examiner has submitted no such affidavit.

5. Attachment for PTO-948

Applicants would like to note that an attachment was included with the Final Office Action mailed 10/23/01. This attachment is entitled "Attachment for PTO-948 (Rev. 03/01, or earlier)" and provides information on how to effect drawing changes. Applicants can find no indication in the present Office Action (mailed 10/23/01) that drawing changes have been requested by the Examiner or Draftsperson and thus, have taken no measures to make any corrections. Should the Examiner have any information to the contrary, that would indicate that drawings changes are required, Applicants respectfully request that the Examiner provide the necessary information as soon as possible.

6. Supplemental Information Disclosure Statement

Applicants are preparing a Supplemental Information Disclosure Statement and will submit it by separate cover.

CONCLUSION

Applicants believe that the arguments set forth above traverse the Examiner's rejections and therefore request that these grounds for rejection be withdrawn for the reasons set forth above. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned collect at (617)-252-3353.

Dated: March 22, 2002

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APPENDIX I

MARKED-UP VERSION OF REWRITTEN CLAIMS

PURSUANT TO 37 C.F.R. § 1.121 (c)(1)(ii)

42. (Amended) A method of constructing a population of altered heavy chain variable region encoding nucleic acids, comprising:

a) providing a representation of first and second reference amino acid sequences, said first reference [nucleic] amino acid sequence comprising the sequence of a donor heavy chain variable region, said donor variable region comprising i) framework regions and ii) three complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor heavy chain variable region comprising framework regions;

[b) synthesizing i) a first population of oligonucleotides, each encoding at least one modified complementarity-determining region, wherein said modified complementarity-determining region comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence; and ii) a second population of oligonucleotides, comprising oligonucleotides encoding modified portions of a heavy chain variable region framework, said modified portion containing a plurality of changed amino acids at one or more positions when compared to said acceptor framework region reference sequence, wherein said framework positions that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence;]

b) synthesizing i) a first population of oligonucleotides, comprising oligonucleotides encoding a modified heavy chain variable region framework region, or portion thereof, wherein said heavy chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework region reference sequence, wherein said framework positions that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence; and ii) a second population of oligonucleotides, each

encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence, and one or more portions of adjacent framework regions which are capable of hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides [under conditions such that at least a portion of said oligonucleotides hybridize] so as to create overlapping oligonucleotides; and

d) treating said overlapping oligonucleotides under conditions such that a population of altered heavy chain variable region encoding nucleic acids is constructed.

47. (Amended) A method of constructing a population of altered light chain variable region encoding nucleic acids, comprising:

a) providing a representation of first and second reference amino acid sequences, said first reference [nucleic] amino acid sequence comprising the sequence of a donor light chain variable region, said donor variable region comprising i) framework regions and ii) three complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference amino acid sequence comprising the sequence of an acceptor light chain variable region comprising framework regions;

[b) synthesizing i) a first population of oligonucleotides, each encoding at least one modified complementarity-determining region, wherein said modified complementarity-determining region comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence; and ii) a second population of oligonucleotides, comprising oligonucleotides encoding modified portions of a light chain variable region framework, said modified portion containing a plurality of changed amino acids at one or more positions when compared to said acceptor framework region reference sequence, wherein said framework positions that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence;]

b) synthesizing i) a first population of oligonucleotides, comprising oligonucleotides encoding a modified light chain variable region framework region, or portion thereof, wherein said light chain variable region framework region, or portion thereof, contains a plurality of changed amino acids at one or more positions when compared to said acceptor framework region reference sequence, wherein said framework positions that are changed are selected from among said acceptor framework positions of said second reference sequence that differ at the corresponding position compared to the donor framework positions of said first reference sequence; and ii) a second population of oligonucleotides, each encoding at least one modified complementarity-determining region, or portion thereof, wherein said modified complementarity-determining region, or portion thereof, comprises a different amino acid at one or more positions when compared to the corresponding donor complementarity-determining region amino acid reference sequence, and one or more portions of adjacent framework regions which are capable of hybridizing to said first population of oligonucleotides; and

c) mixing said first and second populations of oligonucleotides [under conditions such that at least a portion of said oligonucleotides hybridize] so as to create overlapping oligonucleotides; and

d) treating said overlapping oligonucleotides under conditions such that a population of altered light chain variable region encoding nucleic acids is constructed.